the three-heterodimer channel produced no detectable current. Thus, although STIM1 can bind WT-L273D heterodimers, the binding energy provided does not open the channel. Future experiments will test whether L273 acts simply to bind STIM1 or whether it also contributes to coupling binding to channel opening.

1602-Pos Board B332
Mechanism of Activation of Calcium Channel Orai1 by its Regulatory Partner Stim1
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Store-operated calcium entry in T lymphocytes depends on the sensing by STIM1 of cellular calcium store depletion, leading to an interaction of STIM1 with ORAI1 and culminating in calcium flux through the ORAI1 channel. Some important features of the STIM1-ORAI1 interaction have been inferred from studies of mutated channels. A recently published structure of the Drosophila Orai channel, which has high sequence homology with human ORAI1, revealed the resting state pore architecture of the channel and confirmed evidence from biochemical and electrophysiological studies that transmembrane helices 1 line the pore. However, the critical steps of STIM1 binding and the resulting transition of the channel to its active calcium-conducting state are yet to be delineated. Here we demonstrate a STIM1-mediated conformational change in the purified wildtype ORAI1 channel that correlates with calcium flux through the channel reconstituted into liposomes. Introduction of the known disabling mutation R91W blocks the conformational change as well as calcium flux through the reconstituted channel. Mutations in the N-terminal cytoplasmic region of ORAI1 that disrupt STIM1 interaction with this part of ORAI1 are sufficient to abrogate the gating signal, although these mutations do not affect STIM1 interaction with the C-terminal region of ORAI1. Our study offers key insights into the STIM1-dependent gating of the ORAI1 channel.

1603-Pos Board B333
Mechanism of Activation of Store-Operated Calcium Entry by 2-Aminoethoxydiphenyl Borate
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Store-operated Ca^{2+} entry (SOCE) is mediate by STIM-induced activation of Orai channels. The small molecule, 2-aminoethoxydiphenyl borate (2-APB), is known to have a biphasic effect on SOCE; lower 2-APB ($\leq$10 μM) enhances, while higher levels of 2-APB (50-100 μM) are strongly inhibitory following a transient activation. But the mechanism by which 2-APB activates SOCe is still elusive. The effects of 2-APB were examined on coupling between various Orai1 mutants and STIM1, C-terminal (ct) STIM1 fragments, or the STIM-Orai activating region (SOAR) of STIM1, using a combination of imaging and whole-cell patch clamp analysis. Orai1 coupling with STIM1ct, STIM1ct-4EA and SOAR can be transiently activated by 2-APB at low levels (10 μM). The activation of SOCE by low 2-APB is induced by its action on the SOAR-Orai1 complex. We examined which domains/residues in Orai1 or in STIM1 are essential for the activating effect of 2-APB. Adjustment of cytosolic pH upward using nigericin abolished the action of 2-APB. In contrast, lower pH potentiated the activating effect of 2-APB. Our results reveal that the Orai1 C-terminus and N-terminus function in a concerted manner to mediate the activating effect of 2-APB on SOCE. Mutational analysis reveals that the negatively charged residues within the Orai1 C-terminal region are not required for the 2-APB-induced SOCE activation. Our studies provide new insights into the mechanism of 2-APB-induced activation of coupling between STIM and Orai and the triggering of SOCE.

1604-Pos Board B334
Exploring the Role of Pore Waters and Counterions in the Calcium Release-Activated Calcium Channel Conductance with Computation
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Calcium release-activated calcium (CRAC) channels are integral proteins of the plasma membrane, and play a central role in cellular signaling by generating a sustained influx of calcium following its depletion from the intracellular repositories. Previously, we studied the binding of sodium chloride in wild type Orai and its V174A mutant. Our computations showed that even without significant channel-gating motions, a subtle change in the number of pore-waters is sufficient to reshape the local electrostatic field and modulate the energetics of ion conduction. To fully characterize the general permeation pathway of CRAC channels, we further study the flux of monovalent ions through the V174A mutant channel. The presence of a hyperpolarized potential facilitates the passage of ions, so that ''real'' permeation could be observed in the nanosecond timescale MD simulations. Although one can imagine two possible permeation mechanisms for cations to go from the extracellular to the intracellular region, namely either (1) ''direct permeation'', in which cations pass through the hydrophobic region of the pore, in a partially dehydrated state, and then meet anions at the basic region or (2) ''anion-assisted permeation'', in which anions cross over the hydrophobic region in advance, and then accompany the cations during their movement along the central channel. The latter was observed in our MD simulations. Free energy calculations show that Na^+ can easily pass through the central pore with the help of Cl^-, with the major barrier height of ~5 kcal/mol, in the presence of the external field. The functional role of anions we have identified provides a rationale for the presence of the basic region in the cation channel. Our computations thus illustrate how the intrinsic properties of membrane protein design can contribute to channel gating.

1605-Pos Board B335
Ph Dependence of Orai1 and Orai3 Store-Operated Current
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Calcium influx through the Ca^{2+} release-activated Ca^{2+} (CRAC) channel plays a critical role in human T-cell activation. As the prototypical store-operated Ca^{2+} channel, CRAC channel opening requires direct molecular interaction between the endoplasmic reticulum-resident Ca^{2+} sensor STIM1 and plasma membrane pore-forming Orai subunits. Aside from Orai1, mammals express two homologs: Orai2 and Orai3. In this study we found that unlike Orai1, which is blocked by acidic pH and activated by alkaline pH, the Orai3 Stim1-activated current is potentiated by protons. Following current development, replacement of Ringer solution to pH 6.0 alters the I-V shape, inducing a ''bulge'' of inward current at −30 mV. Ion substitution experiments revealed that this current is mostly conducted by Na^+ and is blocked by external Ca^{2+}. Orai1 and Orai3 are identical through TM1 and the critical glutamate that confers Ca^{2+} selectivity; yet only Orai3 exhibits the acid-induced increase of sodium permeability. Using Orai1-Orai3 chimeras we identified several parts of the channel responsible for pH sensitivity. Among the three Orai homologs, Orai1 is unique in being glycosylated. Point mutation of the N223 glycosylation site (N223A) results in acid-induced Na^+ current, suggesting that glycosylation can fine-tune the ion selectivity of Orai1. Orai3 with the internal second loop taken from Orai1 gains alkaline sensitivity but the reciprocal chimera is indistinguishable from Orai1, suggesting that there should be at least two sites responsible for alkaline activation. Orai3 with TM2-loop2-TM3 taken from Orai1 loses acid sensitivity but the reciprocal chimera does not gain it, indicating the presence of at least two sites sensitive to protons. This novel property of Orai3 - the ability of STIM1-operated Na^+ current to be activated by acidic pH - may provide clues for its possible physiological functions.

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Dissecting the Molecular Mechanism of 2-APB-Induced Inhibition of Stim1-Orai1 Coupling
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Store-operated Ca^{2+} entry (SOCe) is a major Ca^{2+} entry pathway in non-excitable cells and is essential in physiological Ca^{2+} signaling and homeostasis. SOCe is mediated by the ER Ca^{2+} sensing STIM proteins and the PM calcium channel, Orai. The aim of this study was to dissect the molecular mechanism of the inhibitory action of the SOCe modifying reagent, 2-aminoethoxydiphenyl borate (2-APB). We demonstrate that although 50 μM 2-APB can block SOCe, it does not prevent STIM1 puncta formation when Orai1 is co-expressed. Neither can it decrease STIM1-Orai1 FRET, thus the inhibitory effect of 2-APB is unlikely to be caused by any ability to block STIM1 puncta formation or physically uncouple STIM1 and Orai1. However, although preincubation of 2-APB does not inhibit Ca^{2+} influx through the action of the constitutively active Orai1-V102C in the absence of expressed STIM1, it can inhibit SOCe mediated by the combination of STIM1 and Orai1-V102C. Therefore, 2-APB preincubation can inhibit SOCe by functionally uncoupling STIM1 and Orai1. We identified one critical residue on STIM1 and several critical residues on the N-terminus of Orai1, both of which could affect functional STIM1-Orai1 coupling and SOCe in a graded manner. To determine possible inhibitory